# Ready Gel® Precast Gels

# Application Guide

Catalog Number 161-0993



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# General Information

#### 1.1 Introduction

Ready Gel<sup>®</sup> precast gels greatly simplify polyacrylamide gel electrophoresis. They are specifically for use with the Mini-PROTEAN Systems (Mini-PROTEAN Tetra, Mini-PROTEAN-III and Mini-PROTEAN Dodeca Cells). Stringent production and quality control criteria, and the use of the highest quality reagents, ensure reproducible electrophoretic analysis with minimum effort. Every gel is checked during production for defects, and each lot of gels is further tested by electrophoresis to verify quality.

Ready Gel precast gels come ready to use with preformed sample wells and a stacking gel when necessary. Each Ready Gel Cassette is 8 x 10 cm (H x W) and 4.0 mm thick. Gel dimension is 6.8 x 8.6 cm (HxW) and 1.0 mm thick. Each gel is individually packaged in a leakproof pouch with an absorbent pad containing gel buffer and 0.02% Sodium Azide.

Ready Gel precast gels are available for use in Tris-glycine (Tris-HCl and zymogram gels), Tris-Tricine, TBE, TBE-urea, and IEF buffer systems. The Tris-HCl gels can be used for SDS-PAGE and non-SDS gel electrophoresis. The Tris-Tricine/peptide gels are optimized for peptide electrophoresis. The TBE gels are for use in nucleic acid electrophoresis and can be used for native protein electrophoresis. TBE-urea gels provide denaturing conditions for nucleic acids. Resolution of different size ranges of proteins or nucleic acids can be obtained by choosing the correct gel.

# 1.2 Mini-format gel System Specifications

Gel material Polyacrylamide Gel dimensions  $8.6 \times 6.8 \text{ cm (W} \times \text{L)}$ 

Gel thickness 1.0 mm
Resolving gel height 5.5 cm

Cassette dimensions 10 x 8.0 cm (W x L)

Cassette material Back (long): acrylic; front (short): glass

Comb material Polycarbonate

Total running buffer volume 700 ml for 2 gels, 1,000 ml for L. gels (Mini-PROTEAN Tetra Cell)

Storage conditions Store flat at 4°C; DO NOT FREEZE

# 1.3 Ready Gel Comb Configurations

# Comb Load Volume 9-well 30 µl

10-well 30 µl 10-well 50 µl 12-well 20 µl 15-well 15 µl

IPG 7 cm ReadyStrip™ IPG strip

Prep 450 µl with one 15 µl reference well

# Setup and Basic Operation Using Mini-PROTEAN Tetra Cell

### 2.1 Setting Up and Running Ready Gel Precast Gels

- 1. Each Ready Gel should be used immediately after it is removed from the storage pouch.
- 2. Remove the comb and gently rinse the wells with deionized water or running buffer.
- 3. Use the key knife or a razor blade to cut the tape at the bottom of the gel along the black "cut here" line. It is helpful to cut all the way to the edge of the cassette where the pull tab begins.
- 4. Pull the tape tab along the cut line, up from the cassette and at an angle towards the comb end of the gel.

## **Required materials:**

- Clean and dry Mini-PROTEAN<sup>®</sup> Tetra cell tank
- Electrophoresis module (Electrode Assembly Module only for 1 or 2 gels; for 3 or 4 gels also use the Companion Running Module)
- Running buffer (700 ml for 2 gels; 1000 ml for 4 gels)
- Ready Gel<sup>®</sup> precast gels or hand-cast gels
- PowerPac<sup>™</sup> Basic power supply

#### 1. Assembly

**Note**: When running 2 gels only, use the Electrode Assembly (the one with the banana plugs), Not the Companion Running Module (the one without the banana plugs). When running 4 gels, both the Electrode Assembly and the Companion Running Module must be used, for a total of 4 gels (2 gels per assembly).

- a. Set the clamping frame to the open position on a clean flat surface (see Figure 4a)
- b. Place the first gel sandwich or gel cassette (with the short plate facing inward) onto the gel supports; gel supports are molded into the bottom of the clamping frame assembly; there are two supports in each side of the assembly. Note that the gel will now rest at a 30° angle, tilting away from the center of the clamping frame. Please use caution when placing the first gel, making sure that the clamping frame remains balanced and does not tip over. Now, place the second gel on the other side of the clamping frame, again by resting the gel onto the supports. At this point there will be two gels resting at an angle, one on either side of the clamping frame, tilting away from the center of the frame (see Figure 4b).

**Note**: It is critical that gel cassettes are placed into the clamping frame with the short plate facing inward. Also, the clamping frame requires 2 gels to create a functioning assembly, If an odd number of gels (1 or 3) is being run, you must use the buffer dam (see Figure 4b).

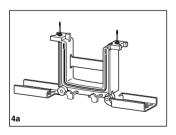
- c. Using one hand, gently pull both gels towards each other, making sure that they rest firmly and squarely against the green gaskets that are built into the clamping frame; make certain that the short plates sit just below the notch at the top of the green gasket.
- d. While gently squeezing the gel sandwiches or cassettes against the green gaskets with one hand (keeping constant pressure and both gels firmly held in place), slide the green arms of the clamping frame over the gels, locking them into place. Alternatively, you may choose to pick-up the entire assembly with both hands, making sure that the gels do not shift, and simultaneously sliding both arms of the clamping frame into place (see Figure 4c).

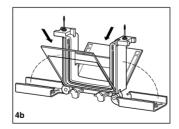
The arms of the clamping frame push the short plates of each gel cassette up against the notch in the green gasket, creating a leak-proof seal (check again to make certain that the short plates sit just below the notch at the top of the green gasket). At this point, the sample wells can be washed-out with running buffer, and sample can be loaded (Figure 4d).

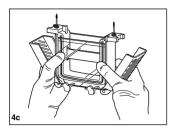
Note: If running more than 2 gels, repeat steps 1a-d with the Companion Running Module.

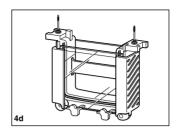
**Important Note**: Do not attempt to lock the green arms of the clamping frame, without first ensuring that the gel cassettes are perfectly aligned and stabilized against the notches on the green gaskets of the module. To prevent the gels from shifting during the locking step, firmly and evenly grip them in place against the core of the module with one hand.

CAUTION: When running 1 or 2 gels only, <u>DO NOT</u> place the Companion Running Module in the tank. Doing so will cause excessive heat generation and prevent electrophoretic separation.









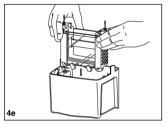


Fig. 4. Assembling the Mini-PROTEAN Tetra Cell Electrophoresis Module.

# Section 3 SDS-PAGE

#### 3.1 Introduction

Ready Gel Tris-HCl gels provide a versatile system for the separation of proteins by molecular weight (SDS-PAGE conditions) or charge to mass ratio (native conditions). (See section 4 for native PAGE applications and protocols.) This is possible because Ready Gel Tris-HCl gels are made without SDS, allowing the sample buffer and running buffer to determine the separation mechanism. Historically, SDS-PAGE systems contained SDS in both the gel and the running buffer. Reproducible SDS-PAGE separations are performed in gels lacking SDS provided the sample buffer and running buffers contain sufficient SDS to saturate the proteins during electrophoresis. The recommended concentration of SDS is 2% in sample buffer and 0.1% in running buffers.

SDS-PAGE uses discontinuous chloride and glycine ion fronts to form moving boundaries that stack and then separate SDS-coated polypeptides by molecular weight. Protein samples are prepared in a reducing denaturing sample buffer containing either 2-mercaptoethanol or dithiothreitol as the reducing reagent, and heat and SDS are used to denature the proteins. 2-Mercaptoethanol and dithiothreitol eliminate protein secondary structure by reducing disulfide bonds. SDS minimizes charge variability among proteins, giving them the same charge to mass ratio and forcing them into rod-like shapes. This effectively eliminates the effects of protein conformation and native charge density on the electrophoretic migration distance. Molecular weight determinations are obtained by plotting the logarithm of protein molecular mass vs. the relative mobility ( $R_{\rm f}$  = distance migrated by protein/distance migrated by dye front).

### 3.2 Ready Gel Tris-HCl Gel Composition

Gel buffer 0.375 M Tris-HCl, pH 8.8

Cross-linker 2.6% C Stacking gel 4% T, 2.6% C

Storage buffer 0.375 M Tris-HCl, pH 8.8

Shelf life 12 weeks

# 3.3 Ready Gel Tris-HCl Gel Selection Guide

Tris-HCl gels are available in a wide selection of single percentages and gradients for the separation of proteins by SDS-PAGE.

Tris-HCI Gels	<b>Optimal Separation</b>	Tris-HCI Gradient Gels	<b>Optimal Separation</b>
5%	100-250 kD	4–15%	20-250 kD
7.5%	40-200 kD	4–20%	10-200 kD
10%	30-150 kD	8–16%	20-120 kD
12%	20-120 kD	10–20%	10-100 kD
15%	10-100 kD		
18%	10-50 kD		

#### 3.4 SDS-PAGE Buffers

Running Buffer	1X Working Concentration	10x Stock
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 25 mM Tris
 Tris base
 15.0 g

 192 mM glycine
 Glycine
 72.0 g

 0.1% SDS
 SDS
 5.0 g

to 500 ml with deionized water

Note: running buffer should be ~ pH 8.3. Do not adjust the pH.

Sample Buffer 2X Working Concentration 2X Stock

62.5 mM Tris-HCl, pH 6.8 0.5 M Tris-HCl, pH 6.8 1.0 ml 2% SDS 10% (w/v) SDS 1.6 ml 25% alvoerol Glycerol 2.0 ml 0.01% Bromophenol Blue 1.0% Bromophenol Blue 0.08 ml 5% 2-mercaptoethanol 2-Mercaptoethanol 0.4 ml or 350 mM DTT (added fresh) Deionized water 2.92 ml 8.0 ml

# 3.5 Sample Preparation

Determine the appropriate protein concentration of your sample based on the detection method and load volume used. (See section 10.1 for approximate stain sensitivities.) Dilute 1 part sample with 1 part sample buffer (see section 3.4) and heat at 95°C for 5 min.

# 3.6 Running Conditions

**Power conditions** 200 V constant

Starting current: 50 mA/gel Final current: 30 mA/gel

Run time 35 min

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# Section 4 Native PAGE

#### 4.1 Introduction

Ready Gel Tris-HCl gels are made without SDS, allowing separation of protein in their native conformation. The nonreducing and nondenaturing environment of native PAGE allows the detection of biological activity and can improve antibody detection. Native PAGE can also be used to resolve multiple protein bands where molecular mass separation by SDS-PAGE would reveal only one.

Native PAGE uses the same discontinuous chloride and glycine ion fronts as SDS-PAGE to form moving boundaries that stack and then separate polypeptides by charge to mass ratio. Proteins are prepared in a nonreducing nondenaturing sample buffer, which maintains the proteins' secondary structure and native charge density. Native PAGE is not suitable for accurate molecular weight determination due to the variability of charge to mass ratio among different proteins.

### 4.2 Ready Gel Tris-HCl Gel Composition

Gel buffer 0.375 M Tris-HCl, pH 8.8

Cross-linker 2.6% C Stacking gel 4% T, 2.6% C

Storage buffer 0.375 M Tris-HCl, pH 8.8, NaN<sub>3</sub> Shelf life 12 weeks from the date of manufacture

# 4.3 Ready Gel Tris-HCl Gel Selection

Native PAGE separates by charge to mass ratio, making individual protein migration protein dependent. Optimal Tris-HCl gel percentages will have to be determined experimentally.

#### 4.4 Native PAGE Buffers

Running Buffer	Working Concentration 25 mM Tris 192 mM glycine	10x Stock Tris base Glycine to 500 ml with deionized water  Note: running buffer should be ~ pH 8.3. Do not adjust the pH.	15.0 g 72.0 g 87.0 g
Sample Buffer	2X Working Concentration 62.5 mM Tris-HCl, pH 6.8 25% glycerol 1% Bromophenol Blue	2X Stock 0.5 M Tris-HCl, pH 6.8 Glycerol 1% Bromophenol Blue Deionized water	1.0 ml 2.0 ml 1.0 ml 4.92 ml 8.0 ml

#### 4.5 Sample Preparation

Determine the desired protein concentration and load volume of your sample based on the detection method used. (See section 10.1 for approximate stain sensitivities). Sample preparation for native PAGE applications requires special consideration. In the absence of SDS, the net charge of a polypeptide will be determined by the pH of the sample buffer. Only polypeptides with a net negative charge will migrate into a native PAGE Tris-HCl gel. Most polypeptides have an acidic or slightly basic pl (~3-8). These proteins can be separated using a standard protocol by diluting 1 part sample with 1 part native sample buffer (see section 4.4: DO NOT HEAT SAMPLES).

Strongly basic peptides (pl >9) will have a net positive charge in a native PAGE Tris-HCl gel. In order for polypeptides with a net positive charge to migrate into a native PAGE Tris-HCl gel, the polarity of the electrodes must be changed by reversing the color-coded jacks when connecting to the power supply.

#### **Running Conditions** 4.6

Power conditions 200 V constant

Starting current:

50 mA/ael Final current: 30 mA/ael

Run time 35 min

# Peptide Analysis

#### 5.1 Introduction

Ready Gel Tris-Tricine/peptide gels are optimized for separating peptides and proteins <10 kD. Superior resolution of peptides is achieved by moving the peptide-SDS complexes more slowly through the gel. This allows the faster moving SDS micelles, which normally interfere with peptide separations, to completely separate from the peptides, allowing distinct peptide bands to resolve.

# 5.2 Ready Gel Tris-Tricine/Peptide Gel Composition

Gel buffer 1.0 M Tris-HCl, pH 8.45

Cross-linker 2.6% C Stacking gel 4% T, 2.6% C

Storage buffer 1.0 M Tris-HCl, pH 8.45, NaN<sub>3</sub> Shelf life 12 weeks from the date of manufacture

# 5.3 Ready Gel Tris-Tricine/Peptide Gel Selection Guide

Tris-Tricine/peptide gels are available in either a single percentage gel or a linear gradient gel.

Peptide Gel	Optimal Separation	
16.5%	15–30 kD	
10-20%	1-40 kD	

#### 5.4 Tris-Tricine/Peptide Buffers

Running buffer Working Concentration 10x Stock

 100 mM Tris
 Tris base
 60.55 g

 100 mM Tricine
 Tricine
 89.60 g

 0.1% SDS
 SDS
 5.0 g

to 500 ml with deionized water

Note: Tricine running buffer should be ~ pH 8.25. Do not adjust the pH.

Sample Buffer Working Concentration 2X Stock

200 mM Tris-HCl, pH 6.8 1.0 M Tris-HCl, pH 6.8 2.0 ml 2% SDS 10% SDS 2.0 ml 40% alycerol Glycerol 4.0 ml 0.04% Coomassie Blue G-250 0.5% Coomassie Blue G-250  $0.8 \, \text{ml}$ 2% 2-mercaptoethanol 2-Mercaptoethanol 0.2 ml or 350 mM DTT (Added fresh) Deionized water 1.0 ml 10.0 ml

### 5.5 Sample Preparation

Determine the appropriate protein concentration of your sample based on the detection method and load volume used. (See section 10.2 for approximate stain sensitivities.) Dilute 1 part sample with 1 part sample buffer and heat at 95°C for 5 min.

# 5.6 Running Conditions

**Power Conditions** 100 V constant

Starting current: 30–35 mA/gel Final current: 15–20 mA/gel

Run Time 100 min

# Isoelectric Focusing

#### 6.1 Introduction

Ready Gel IEF gels are cast with Bio-Rad's Bio-Lyte<sup>®</sup> ampholytes, amphoteric molecules that set up a pH gradient across the gels. Proteins migrate in IEF gels to their neutral isoelectric point (pl), where the protein has zero net charge. Ready Gel IEF gels contain no denaturing agents, so all focusing is performed under native conditions.

## 6.2 Ready Gel IEF Gel Composition

Gel buffer 2% ampholyte, pH 3-10, 5-8

Cross-linker 3.0% C Stacking gel None

Storage buffer Deionized water, NaN<sub>3</sub>

Shelf life 26 weeks from the date of manufacture

### 6.3 Ready Gel IEF Gel Selection Guide

Ready Gel IEF gels are available in narrow and broad pH ranges.

IEF gel	pH Range
5–8	5–8
3–10	4-8.5

#### 6.4 IEF Buffers

**Running buffer** 1x Cathode Buffer 5x Cathode Buffer

20 mM lysine (free base)

Lysine (free base)

Lysine (free base)

14.50 g

20 mM arginine (free base)

Arginine (free base)

17.42 g

to 1 L with deionized water

<u>1x Anode Buffer</u> <u>10x Anode Buffer</u>

7mM% phosphoric acid Phosphoric acid 4.2 ml

to 1 L with deionized water

Sample Buffer 50% glycerol

### 6.5 Sample Preparation

Determine the appropriate protein concentration of your sample based on the detection method and load volume used. (See section 10.3 for approximate stain sensitivities.) Dilute 1 part sample with 1 part sample buffer.

# 6.6 Running Conditions

Power conditions Stepwise

 100 V constant
 60 min

 250 V constant
 60 min

 500 V constant
 30 min

 Starting current:
 5–15 mA/gel

 Final current:
 5–15 mA/gel

Run time 150 min

# Protease Analysis by Zymogram PAGE

#### 7.1 Introduction

Ready Gel zymogram gels are used to test for proteolytic activity when performing protein characterizations. Gels are cast with gelatin or casein, which act as substrates for proteases that are separated on the gel. Proteases are detected by renaturing the enzyme followed by a development period in which the protease breaks down the substrate. Zymogram gels are stained with Coomassie Blue R-250, which stains the substrate while leaving clear areas around active proteases.

# 7.2 Ready Gel Zymogram Gel Composition

Gel buffer 0.375 M Tris-HCl, pH 8.6

Cross-linker 2.6% C Stacking gel 4% T, 2.6% C

Storage buffer 0.375 M Tris-HCl, pH 8.6, 0.2% NaN<sub>3</sub> Shelf life 12 weeks from the date of manufacture

# 7.3 Ready Gel Zymogram Gel Selection Guide

Ready Gel zymogram gels are available with either gelatin or casein as substrate and should be selected based on their substrate and separation range.

Zymogram Gel	Optimal Separation	
10% zymogram gel with gelatin	30-150 kD	
12% zymogram gel with casein	20-120 kD	

#### 7.4 Zymogram Buffers

Running buffer Working Concentration 10x Stock

 25 mM Tris
 Tris base
 15.0 g

 192 mM glycine
 Glycine
 72.0 g

 0.1% SDS
 SDS
 5.0 g

to 500 ml with deionized water

Note: running buffer should be ~pH 8.3. Do not adjust the pH.

Sample Buffer Working Concentration 2X Stock

## 7.5 Sample Preparation

Determine the appropriate protein concentration of your sample based on the detection method and load volume used. (See section 10.4 for approximate stain sensitivities.) Dilute 1 part sample buffer with 1 part Zymogram sample buffer. Dry samples can be dissolved directly in sample buffer. Do not heat.

# 7.6 Running Conditions

**Power conditions** 100 V constant

Starting current: 10–15 mA/gel Final current: 6 mA/gel

Run time 90 min

# Nondenaturing Nucleic Acid PAGE

#### 8.1 Introduction

Ready Gel TBE gels are ideal for separating small dsDNA fragments, especially PCR products. The uniform nature of DNA molecules provides samples with near-uniform charge to mass ratio, allowing nondenaturing nucleic acid PAGE to separate dsDNA by mass using a continuous TBE buffer system.

# 8.2 Ready Gel TBE Gel Composition

Gel buffer 89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3

Cross-linker 3.3% C Stacking gel 4% T, 3.3% C

Storage buffer 89 mM Tris, 89 mM boric acid, 2 mM EDTA, NaN<sub>3</sub>

Shelf life 12 weeks from the date of manufacture

### 8.3 Ready Gel TBE Gel Selection Guide

Ready Gel TBE gels are available in a selection of single percentages and gradients for the separation of dsDNA.

TBE Gels	<b>Optimal Separation</b>	TBE Gradient Gels	<b>Optimal Separation</b>
5%	200-2,000 bp	4–20%	10-2,000 bp
10%	50-1,500 bp		
15%	20-1,000 bp		

#### 8.4 Nondenaturing Nucleic Acid PAGE Buffers

Running Buffer Working Concentration 10x Stock

 50 mM Tris
 Tris base
 0.06 g

 89 mM boric acid
 Boric acid
 27.5 g

 5 mM EDTA
 0.5 M EDTA (pH 8.0)
 0.1 ml

to 500 ml with deionized water Note: TBE running buffer should be  $\sim$  pH 8.3. Do not adjust the pH.

Sample Buffer 2X Working Concentration

 50 nM EDTA
 Tris Base
 0.06 g

 25% glycerol
 0.5 M EDTA
 0.1 ml

 0.2% Bromophenol Blue
 Glycerol
 2.5 ml

 0.2% Xylene Cyanole FF
 1% Bromophenol Blue
 2.0 ml

 1% Xylene Cyanole FF
 2.0 ml

Make up to 10 ml with deionized water

### 8.5 Sample Preparation

Determine the desired DNA concentration of your sample based on the detection method used. (See section 10.5 for approximate stain sensitivities.) Dilute 1 part sample with 4 parts sample buffer (see section 8.4).

### 8.6 Running Conditions

**Power conditions** 100 V constant

Starting current: 13 mA/gel Final current: 11 mA/gel

Run time 45–105 min

# Denaturing Nucleic Acid PAGE

#### 9.1 Introduction

Ready Gel TBE-urea gels are ideal for separating small ssDNA and RNA fragments. Applications include oligonucleotide analysis, RNase protection assays, and northern blotting.

# 9.2 Ready Gel TBE-Urea Gel Composition

Gel buffer 89 mM Tris, 89 mM boric acid, 2 mM EDTA, 7 M urea, pH 8.3

Cross-linker 3.3% C

Stacking gel 4% T, 3.3% C

Storage buffer 89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3,  $NaN_3$ 

Shelf life 8 weeks from the date of manufacture

# 9.3 Ready Gel TBE-Urea Gel Selection Guide

Ready Gel TBE-urea gels are available in a range of single percentage gels.

TBE-Urea	Optimal Separation
5%	50-1,000 bases
10%	25-300 bases
15%	10-50 bases

#### **TBE-Urea Buffers** 9.4

**Running Buffer** Working Concentration 10x Stock

> 89 mM Tris Tris base 54.0 a 89 mM horic acid Boric acid 27.5 2 mM FDTA 0.5 M EDTA (pH 8.0) 20.0 ml

> > to 500 ml with deionized water

Sample Buffer Working Concentration

> 89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0 10x TBF

12% FicoII

1.2 a 0.01% Bromophenol Blue Urea 4.2 a 0.02% Xvlene Cvanole FF 1% Bromophenol blue  $0.1 \, \text{ml}$ 7 M urea 1% Xvlene Cvanole FF 0.2 ml

FicoII

0.5 M FDTA  $0.02 \, \text{ml}$ 

1 0 ml

Make up to 10 ml with deionized water

#### 9.5 **Sample Preparation**

Determine the desired ssDNA or RNA concentration for your sample based on the detection method used. (See section 10.6 for appropriate stain sensitivities.) Dilute 1 part sample with 1 part TBE-urea sample buffer. Dry samples can be dissolved directly in sample buffer. Heat to 70-90°C 4 min before loading.

#### **Running Conditions** 9.6

Power conditions 200 V constant

> Starting current: 15 mA/ael Final current: 10 mA/ael

Run time 40-70 min

# Section 10 Detection

#### 10.1 SDS-PAGE and Native PAGE Detection

#### **Total Protein Gel Stains**

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Method	Sensitivity	Optimal Protein Load	Advantages	Disadvantages
Coomassie Blue R-250	36-47 ng	~0.5 µg/band	Laboratory standard	Requires MeOH
Bio-Safe <sup>™</sup> Coomassie stain	8–28 ng	~0.5 µg/band	Nonhazardous, uses no MeOH	More steps than Coomassie Blue R-250
Copper stain	6–12 ng	~0.2 µg/band	Fast, reversible stain be photographed; SDS-PAGE only	Negative stain, must
Zinc stain	6–12 ng	~0.2 µg/band	High-contrast, fast, reversible stain SDS-PAGE only	Negative stain, must be photographed;
Silver Stain <sup>TM</sup> Plus kit	0.6–1.2 ng	~0.01 µg/band	Simple, robust, mass spectrometry compatible	Will not stain glycoproteins
Silver stain	0.6–1.2 ng	~0.01 µg/band	Stains complex proteins, i.e., glycoproteins and lipoproteins	Not mass spectrometry compatible
SYPRO Orange protein stain	4–8 ng	~0.2 µg/band	Will not stain nucleic acids; mass spectrometry compatible	Optimization required for maximum sensitivity
SYPRO Ruby protein gel stain	1–10 ng	~0.2 µg/band	Broad dynamic range, simple robust protocol maximum sensitivity	Requires imaging instrument for
Flamingo Fluorescent gel stain	0.25–0.5 ng	~0,2 ng/band	Broad dynamic range mass spec compatible	Requires imaging instrument for maximum sensitivity

Total Protein Blot Stains				
Method	Sensitivity	Optimal Protein Load	Advantages	Disadvantages
SYPRO Ruby protein blot stain	2–8 ng	~0.2 µg/band immunological procedures	Compatible with mass spectrometry, Edman-based sequencing, and standard maximum sensitivity	Multiple step protocol; Requires imaging instrument for
Colloidal gold stain	1 ng	~0.1 µg/band	Sensitive, one step	Not compatible with nylon membranes
Enhanced colloidal gold detection kit	10-100 pg	~0.1 µg/band	Increases sensitivity of colloidal gold stain	Multiple steps
AmidoBlack 10B	100–1,000 ng	~5 µg/band	Standard membrane stain, economical	Low sensitivity
Immunoblot Detection				
Method	Sensitivity	<b>Optimal Protein Load</b>	Advantages	Disadvantages
4CN colorimetric (HRP)	500 pg	~0.25 µg/band	Fast detection	Results may fade
DAB colorimetric (HRP)	500 pg	~0.25 µg/band	Fast detection	Contains toxic chemicals
Opti-4CN colorimetric (HRP)	100 pg	~0.05 µg/band	Color does not fade	More expensive than 4CN
Amplified Opti-4CN colorimetric (HRP)	10 pg	~0.005 µg/band	High sensitivity, low background	Amplification requires additional steps
BCIP/NBT colorimetric (AP)	100 pg	~0.05 µg/band	Sensitive, multiple antigen	May detect endogenous enzyme activity
Amplified alkaline phosphatase	10 pg	~0.005 µg/band	High sensitivity	Amplification requires additional steps
Immun-Star <sup>™</sup> chemiluminescent (AP)	10 pg	~0.005 µg/band	Long-lasting signal, short and multiple exposures possible	Requires visualization on film or instrumentation
Immun-Star <sup>™</sup> chemiluminescent (hrp)	1–3 pg	~0.005 µgband	Intensifies signal output, very sensitive	Requires visualization on film or instrumentation
Immun-Star WesternC (HRP)	10 fg	~0.005 µgband	long-lasting signal short and multiple exposures possible	Requires visualzation on on film or instrumentation

### 10.2 Peptide Gel Staining

Peptides and small proteins are prone to diffusion and loss during staining. The following protocol uses a fixation step to prevent sample loss and is suitable for detection of bands as low as 10–20 ng.

#### **Fixative Solution**

40% methanol 10% acetic acid

#### Coomassie Brilliant Blue G-250 Stain Solution

0.025% Coomassie Blue G-250 10% acetic acid

#### **Destain Solution**

10% acetic acid

Place gels in fixative solution and equilibrate for 30 min. Stain gels with Coomassie Brilliant Blue G-250 stain solution for 1 hr. Stain should only be used once. Reuse of stain could result in loss of sensitivity. Destain gels 3 times for 15 min or until the desired background is achieved. Some peptides may not be completely fixed and may diffuse out of the gels if fixing and staining times are greatly exceeded.

# 10.3 IEF Gel Staining

Samples on IEF gels can be detected using multiple methods. Use the following table as a guide to select an appropriate staining method.

Method	Sensitivity	Optimal Protein Load	Advantages	Disadvantages
IEF stain	40-50 ng	~0.5 µg/band	Simple, no fixation required	Requires MeOH
Silver Stain Plus kit	0.6–1.2 ng	~0.01 µg/band	Simple, robust, mass spectrometry compatible	Requires TCA fixation
Silver stain	0.6–1.2 ng	~0.01 µg/band	Stains complex proteins, i.e. glycoproteins and lipoproteins	Requires TCA fixation

### 10.4 Zymogram Gel Staining

Prior to staining zymogram gels, sample proteases must be first renatured and allowed to break down the substrate contained in the gel. The following protocol provides basic guidelines for detection. Optimal results should be determined empirically.

#### **Renaturing Solution**

2.5% Triton X-100

#### **Development Solution**

50 mM Tris 200 mM NaCl 5 mM CaCl<sub>2</sub> (anhydrous) 0.02% Brij-35 Adjust to pH 7.5

#### **Staining Solution**

40% methanol 10% acetic acid 0.5% Coomassie Blue R-250

#### **Destaining Solution**

40% methanol 10% acetic acid

Proteins must be renatured first by placing the gels in renaturing solution for 30 min at room temperature. Incubate gels in development solution at 37°C for a minimum of 4 hr. Highest sensitivity is typically achieved with overnight incubation. Optimal results should be determined empirically. Stain gels with Coomassie Brilliant Blue R-250 staining solution for at least 1 hr at room temperature. Destain until clear bands appear against the blue background, approximately

30-60 min.

# 10.5 TBE Gel Staining

Use the following table as a guide to select an appropriate staining method.

Method	Sensitivity	Optimal Protein Load	Advantages	Disadvantages
Ethidium bromide	50 ng	~0.10 µg/band	Classic fluorescent DNA stain	Carcinogenic
Silver stain	1.0–2.0 ng	~0.5 µg/band	More sensitive than ethidium bromide	Multiple steps

# 10.6 TBE-Urea Gel Staining

Samples on denaturing nucleic acid gels can be detected using multiple methods. Use the following table as a guide to select an appropriate staining method.

Method	Sensitivity	Optimal Protein Load	Advantages	Disadvantages
Ethidium bromide Radiant <sup>®</sup> Red	50 ng 10 ng	~0.10 µg/band ~0.10 µg/band	Classic fluorescent DNA stain Fast single-step protocol	Carcinogenic RNA and ssDNA
	· ·			only
Silver stain	1.0–2.0 ng	~0.5 µg/band	More sensitive than ethidium bromide	Multiple steps

# Stock and Staining Solutions

#### 11.1 Stock Solutions

#### 0.5 M Tris-HCl, pH 6.8

6.06 g Tris base

~60 ml deionized water

Adjust to pH 6.8 with HCl. Make to 100 ml with deionized water and store at 4°C.

#### 10% SDS

Dissolve 1 g SDS in water with gentle stirring and bring to 10 ml with deionized water.

#### 1% Bromophenol Blue

Dissolve 0.1 g of Bromophenol Blue in 10 ml deionized water with gentle stirring.

#### 1% Xylene Cyanole FF

Dissolve 0.1 g of Xylene Cyanole FF in 10 ml deionized water with gentle stirring.

#### 0.5 M EDTA

18.6 g of EDTA

~ 50 ml of deionized water

Adjust to pH 8.0 with 1 N NaOH. Make to 100 ml with deionized water and store at 4°C.

### 11.2 Protein Staining Solutions

#### Coomassie Blue R-250 Staining Solution (0.1%)

# Final Concentration

 Methanol
 400 ml
 40%

 Acetic acid
 100 ml
 10%

 Coomassie Blue R-250
 1.0 g
 0.1%

Deionized water 500 ml

Dissolve Coomassie R-250 in methanol/acetic acid. Add deionized water to a final volume of 500 ml.

#### Coomassie Blue R-250 Destaining Solution

#### Final Concentration

 Methanol
 400 ml
 40%

 Acetic acid
 100 ml
 10%

Deionized water 500 ml

### **Silver Staining**

See Bio-Rad's silver stain (catalog #161-0443) or Silver Stain Plus kit (catalog #161-0449) instructions.

#### **IEF Staining Solution**

	Final Cond	entration
Isopropyl alcohol	270 ml	27%
Acetic acid	100 ml	10%
Coomassie Blue R-250	0.4 g	0.04%
Crocein Scarlet	0.5 g	0.05%
Deionized water	630 ml	

#### **IEF Destaining Solution**

	Final Conc	entration
Methanol	400 ml	40%
Acetic acid	100 ml	10%
Deionized water	500 ml	

# 11.3 Peptide Staining Solutions

#### **Fixative Solution**

	Final Conc	entration
Methanol	400 ml	40%
Acetic acid	100 ml	10%
Deionized water	500 ml	

#### Coomassie Blue G-250 Staining Solution (0.025%)

	Final Cond	entration
Acetic acid	100 ml	10%
Coomassie Blue G-250	0.25 g	0.025%
Deionized water	900 ml	

#### Coomassie Blue G-250 Destaining Solution

	Final Conc	entration
Acetic acid	100 ml	10%
Deionized water	900 ml	

# 11.4 Zymogram Staining Solutions

#### Renaturation Buffer

	Final Conc	entration
Triton X-100	25 g	2.5 %
Deionized water	900 ml	
to 1 L with deionized w	ater ater	

#### **Development Solution**

	Final Conc	entration
Tris base	6.06 g	50 mM
NaCl	11.7 g	200 mM
CaCl <sub>2</sub> (anhydrous)	0.56 g	5 mM
30% Brij-35	0.67 ml	0.02%

Dissolve in 900 ml deionized water, adjust to 7.5 with 6 N HCl, make to 1 L with deionized water.

#### **Staining Solution**

	Final Cond	entration
Methanol	400 ml	40%
Acetic acid	100 ml	10%
Coomassie Blue R-250 Deionized water	5 g 500 ml	0.5%

Dissolve Commassie R-250 in Methnaol/acetic acid. Add deionized water to final volume of 500 ml.

# 11.5 Nucleic Acid Staining Solutions

#### **Ethidium Bromide Staining**

Use Bio-Rad's ethidium bromide tablets or ethidium bromide solutions (catalog #161-0430 or 161-0443) for nucleic acid staining solutions.

#### **Silver Staining**

See instructions for Bio-Rad's silver stain, catalog #161-0443, or Silver Stain Plus kit, catalog #161-0449.

#### Radiant® Red Staining

Use Radiant Red stain, catalog #170-3122, for RNA staining.

# Troubleshooting

Improper storage of Ready Gel precast gels can produce numerous artifacts. Gels should be stored flat at 4°C. Avoid freezing or prolonged storage above 4°C. If you suspect your gels have been stored improperly, DO NOT USE THEM.

Problem	Possible Cause	Solution
Samples do not migrate into gel	Tape at the bottom of the cassette not removed	Remove tape
	Insufficient buffer in integral buffer chamber	Fill buffer chamber with 125 ml running buffer
	Insufficient lower electrode buffer	Fill both halves of the lower buffer tank with sufficient running buffer
	Electrical disconnection	Check electrodes and connections
Bands "smile" across gel, band pattern	Excess heating of gel	Check buffer composition
curves upward at both sides of the gel		Completely fill both halves of the lower buffer tank with sufficient running buffer
		Do not exceed recommended running conditions
Skewed or distorted bands, lateral band spreading	Excess salt in samples	Remove salts from sample by dialysis or desalting column prior to sample preparation
	Insufficient sample buffer or wrong formulation	Check buffer composition and dilution instructions
Problem	Possible Cause	Solution
Vertical streaking	Overloaded samples	Dilute sample

		the sample
	Sample precipitation	Centrifuge samples to remove particulates prior to sample loading
Gels run too fast, provide poor resolution, and gel temperature is too high	Running buffer is too concentrated	Check buffer composition
Artifact bands at ~60-70 kD	Possible skin keratin contamination Clean all dishware and wear gloves while handling and loading gel	
		Filter all solutions through nitrocellulose

Selectively remove predominant protein in

Use 10% iodoacetamide to eliminate

keratin bands

# Section 13 Ordering Information

# 13.1 Ready Gel Precast Gels

Ready Gel Tris-HCl Gels	10-Well 30 µl	15-Well 15 µl	Prep Well 450 µl	10-Well 50 µl	12-Well 20 μl	9-Well 30 µl	IPG Comb 7 cm IPG Strip
5% Tris-HCI	161-1210	161-1211		161-1213	161-1214		
7.5% Tris-HCl	161-1100	161-1118	161-1136	161-1154	161-1172		
10% Tris-HCl	161-1101	161-1119	161-1137	161-1155	161-1173	161-1191	161-1390
12% Tris-HCl	161-1102	161-1120	161-1138	161-1156	161-1174		161-1391
15% Tris-HCl	161-1103	161-1121	161-1139	161-1157	161-1175		
18% Tris-HCl	161-1216	161-1217		161-1219	161-1220		
4-15% Tris-HCI	161-1104	161-1122	161-1140	161-1158	161-1176	161-1194	161-1392
4-20% Tris-HCI	161-1105	161-1123	161-1141	161-1159	161-1177		161-1393
8-16% Tris-HCI	161-1222	161-1223		161-1225	161-1226		161-1394
10-20% Tris-HCl	161-1106	161-1124	161-1142	161-1160	161-1178		161-1395
Ready Gel TBE Precast Gels							
5%, TBE	161-1109	161-1127	_	161-1163	161-1181		
10%, TBE	161-1110	161-1128	_	161-1164	161-1182		
15%,TBE	161-1228	161-1229	_		161-1232		
4-20%, TBE	161-1234	161-1235	_	161-1237			
Ready Gel Tris-Tricine/Pe	eptide Preca	st Gels					
16.5% Tris-Tricine/Peptide	161-1107	161-1125	161-1143	161-1161	161-1179	161-1197	
10-20% Tris-Tricine/Peptide	161-1108	161-1126	161-1144	161-1162	161-1180	161-1198	

#### **Ready Gel IEF Precast Gels**

 IEF pH 3-10
 161-1111
 161-1129
 161-1165

 IEF pH 5-8
 161-1112

Ready Gel Zymogram Precast Gels

10% Zymogram, gelatin 161-1113 161-1131 — 161-1167 161-1185

12.5% Zymogram, casein 161-1114 – 161-1168

Ready Gel TBE-Urea Precast Gels

5% TBE-Urea 161-1115 161-1133 —

10% TBE-Urea 161-1116 161-1134

15% TBE-Urea 161-1117 161-1135 161-1189

#### 13.2 Buffers

#### **Premixed Running Buffers**

161-0732	10x Tris/Glycine/SDS, 1 L
161-0772	10x Tris/Glycine/SDS, 5 L
161-0734	10x Tris/Glycine, 1 L
161-0771	10x Tris/Glycine, 5 L
161-0744	10x Tris/Tricine/SDS, 1 L
161-0761	10x IEF Anode Buffer, 250 ml
161-0762	10x IEF Cathode Buffer, 250 ml
161-0733	10x Tris/Boric Acid/EDTA, 1 L
161-0770	10x Tris/Boric Acid/EDTA, 5 L
161-0765	Zymogram Renaturation Buffer, 125 ml
161-0766	Zymogram Development Buffer, 125 m

#### **Individual Reagents**

Tris, 1 kg
Tris, 500 g
Glycine, 250 g
Glycine, 1 kg
Glycine, 2 kg
SDS, 100 g
2-Mercaptoethanol, 25 ml
Dithiothreitol, 1 g
Dithiothreitol, 5 g
Bromophenol Blue, 10 g

# **Premixed Sample Buffers**

161-0737	Laemmli Sample Buffer, 30 ml*
161-0738	Native Sample Buffer, 30 ml
161-0739	Tricine Sample Buffer, 30 ml
161-0763	IEF Sample Buffer, 30 ml
161-0764	Zymogram Sample Buffer, 30 ml
161-0767	Nucleic Acid Sample Buffer, 5x, 10 ml
161-0768	TBE-Urea Sample Buffer, 30 ml

<sup>\*</sup> Requires addition of 2-mercaptoethanol or DTT

# 13.3 Detection Reagents

#### **Total Protein Gel Stains**

161-0436	Coomassie Blue R-250 Stain Solution, 1 L
161-0438	Coomassie Blue R-250 Destain Solution, 1 L
161-0400	Coomassie Brilliant Blue R-250, 10 g
161-0786	Bio-Safe Coomassie Stain, 1 L
161-0470	Copper Stain and Destain Kit
161-0440	Zinc Stain and Destain Kit
161-0449	Silver Stain Plus Kit
161-0443	Bio-Rad Silver Stain Kit
170-3120	SYPRO Orange Protein Stain, 500 µl
170-3125	SYPRO Ruby Protein Gel Stain, 1 L
161-0490	Flamingo Fluorescent Gel Stain (10x), 20 ml
161-0491	Flamingo Fluorescent Gel Stain (10x), 100 ml
161-0492	Flamingo Fluorescent Gel Stain (10x), 500 ml

#### **Immunoblot Detection**

170-6431	HRP Conjugate Substrate Kit, 4CN
170-6535	HRP Color Development Reagent, DAB
170-8238	Amplified Opti-4CN Kit
170-8235	Opti-4CN Substrate Kit
170-6432	BCIP/NBT AP Conjugate Substrate Kit
170-6412	Amplified Alkaline Phosphatase Kit
170-5012	Immun-Star <sup>™</sup> Substrate Pack

#### **Total Protein Blot Stains**

170-3127	SYPRO Ruby Protein Blot Stain, 200 ml
170-6527	Colloidal Gold Total Protein Stain, 500 ml
170-6517	Enhanced Colloidal Gold Detection Kit
161-0402	Amido Black 10B, 25 g

#### 13.4 **Blotting Membranes** 162-0232 0.2 um Nitrocellulose/Filter Paper Sandwich, 8.5 x 13.5 cm, 20 pack 162-0233 0.2 um Nitrocellulose/Filter Paper Sandwich, 8.5 x 13.5 cm, 50 pack 162-0234 0.45 um Nitrocellulose/Filter Paper Sandwich, 8.5 x 13.5 cm, 20 pack 162-0235 0.45 um Nitrocellulose/Filter Paper Sandwich, 8.5 x 13.5 cm, 50 pack 162-0236 Sequi-Blot PVDF/Filter Paper Sandwich, 8.5 x 13.5 cm, 20 pack Sequi-Blot PVDF/Filter Paper Sandwich, 8.5 x 13.5 cm, 50 pack 162-0237 13.5 **Protein and DNA Standards** 161-0363 Precision Plus Protein<sup>™</sup> Unstained Standards (10-250 kD), 1.500 µl, 150 applications 161-0373 Precision All Blue Prestained Standards (10-250 kD), 500 ul. 50 applications Kaleidoscope<sup>™</sup> Prestained Standards, 500 µl, 50 applications 161-0324 Polypeptide SDS-PAGE Standards (1.4-26.6 kD), 200 µl, 400 applications 161-0326 IEF Standards, pl range 4.45-9.6, 250 µl, 500 applications 161-0310 161-0375 Precision Plus Protein<sup>™</sup> Kalaidoscope Standards 500 µl. 50 applications Precision Plus Protein<sup>™</sup> WesternC Standards, 250 µl, 50 applications 161-0370

20 bp EZ Load<sup>™</sup> Molecular Ruler (20–1,000 bp), 50 µg, 100 applications

100 bp EZ Load Molecular Ruler (100-1.000 bp), 25 ug, 100 applications

AmpliSize® Molecular Ruler (50-2.000 bp), 25 µg, 50 applications

Mini-PROTEAN Tetra Cell for Ready Gel Precast gels

100 bp PCR EZ Load Molecular Ruler (100-3,000 bp), 40 ug, 100 applications

# 13.6 Equipment

170-8351 170-8352

170-8353

170-8200

165-8004

165-3302	Mini-PROTEAN 3 Electrophoresis Module
170-3930	Mini Trans-Blot® Electrophoresis Transfer Cell

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# **Catalog Number** 161-0993





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